YEASTS ASSOCIATED WITH CERTAIN BARK BEETLES

PROGRESS REPORT

by

MOSHE SHIFRINE

NOVEMBER 1, 1952

YEAST ASSOCIATED WITH CERTAIN BARK BEETLES

Progress Report

by Moshe Shifrine

INTRODUCTION

The greatest timber loss in California is caused by bark beetles. Since pines are California's main crop tree, the following four species of beetles killing pines are of the utmost importance (Keen 1951).

(1) Dendroctonus brevicomis, western pine beetle, attacks ponderosa and Coulter pines. (Pinus ponderosa and Pinus coulteri)

(2) Dendroctonus monticolae, mountain pine beetle, attacks sugar, western white and ponderosa pines. (P. lambertiana, P. monticola and P. ponderosa)

(3) Dendroctonus jeffreyi, jeffrey pine beetle, attacks only Jeffrey pines. (P. jeffreyi)

(4) Species of Ips, pine engraver, attacks all pines.

The loss of pine timber resulting from bark beetle attacks in California, Washington and Oregon states was estimated at approximately 15,545,000,000 board feet for the period 1931-1940. For California alone, for the year 1951 losses were reported at 400,000,000 board feet. The estimated loss for 1952 is even greater than that for the 1951 period (Callaham 1952).

The beetles bore through the bark of the attacked tree and tunnel in to the phloem on which they feed. The entrance holes, the adult's and larval galleries serve to expose the wood to the air. The outer rings of the tree lose the capacity to conduct solutions successively from the first ring toward the center of the tree. This cuts off the supply of water to the leaves which depletes the top of the tree of water and the tree dies. Failure of the outer rings to conduct solutions is

intimately associated with drying of the wood. This is also closely associated with the penetration of the wood by Ceratostomella pini, a blue-staining fungus, which penetrates deeply into the wood during the early stages of attack, filling the xylem tissues with a mass of mycelium. It was experimentally shown that C. pini growing alone is able to kill the trees (Caird 1934).

In all of the species the first evidence of attack on living trees is the presence of pitch tubes or boring dust on the trunks. Later, the fading yellowish, or reddish, condition of the foliage is conspicuous evidence of the bark beetles' destructive work. All of the studies on attacks by Dendroctonus beetles have demonstrated their ability to attack certain apparently healthy trees and kill them wherever the individuals of a species occur in sufficient numbers to overcome the resistance of the tree (Hopkins 1909).

The association of yeasts with bark beetles is of common occurrence and has been reported by many workers (Person 1929,, Struble 1930, and Holst 1936). There are two theories of tree selection by western bark beetles. In both theories yeasts play a predominant role. The attraction that results in the complete attack of a tree includes two stages. The second stage is common in both theories of tree selection. The first theory maintains that the initial stage of attraction is a result of some volatile substance being produced by the tree. According to the second theory the first beetles to attack a tree do so randomly without any preference. In the second stage, 3-10 days after the first attack, a much more definite attraction is evident. This is a result of phloem fermentation, by the yeasts brought in with the beetles, the end products of which attract the beetles en-masse. That fermented phloem attracts beetles has been shown experimentally (Gordon 1933). The result of the mass attack is the death of the attacked tree in about six weeks.

The ~~two~~ present-day hypotheses of host ~~selection are~~ specificity are based ~~firstly~~ on oleoresin production as a means of host resistance and ~~the~~ second ~~one~~ emphasizes the role of yeasts introduced into the trees. The ~~first~~ former hypothesis is being worked on at the University of California in Berkeley and the ~~second~~ latter is the subject of this paper.

LITERATURE

"Probably the first observer to record the presence of living microorganisms in insects was Raimbert in 1869, who obtained anthrax bacilli from flies exposed to the cadavers of infected animals" (Steinhaus 1946). Since then many articles have appeared covering different aspects of this association. The nutritional study of insects with special reference to microorganisms and their substrata was covered in detail by Baumberger (1919) and Uvarov (1929). Leach (1940), Wingard (1925), Fawcett (1929) and Wallace (1932) discussed the insect transmission of yeasts which cause plant diseases. Steinhaus (1946) published a general review of insect microbiology. Mrak and Phaff (14) in their review of yeasts covered their association with many insects. El-Tabey (1950) covered the association of yeasts with Drosophila flies. The association of yeasts with wood-boring beetles has been reported by many workers. Schneider-Orelli (1913) stated that yeasts were always present in the tunnels of ambrosia beetles. Beck (1922) described a new species of yeast Endomyces bisporus, which she found associated with the bark beetle Ips typographus attacking spruce and fir in Austria. In 1939 this was again found on the same beetles and trees in Poland by Siemaszko (1939). Verral (1944) isolated two yeast species which he named Endomyces bispora and Monilia brunnea, the former being probably the same yeast as described by Beck in 1922 and Stelling Dekker in 1931 and was, therefore, by no means, a new species. Grossman (1930), in her work on the association of bark beetles and blue-stain fungi, regularly isolated yeasts which she did not identify. Leach, Orr and Christensen (1934) found characteristic yeasts constantly associated with bark beetles and the blue-staining fungi in the felled Norway Pine timber. In 1936, Holst isolated a yeast that has been found generally associated with the bark beetles belonging to Dendroctonus and Ips genera. The yeast was subsequently named Zygosaccharomyces pini n. sp. (now known as Z. pastori.). Studying the association of bark beetles and blue staining fungus, Rumbold (1941) observed the presence of Z. pini and several Candida species in two species of Dendroctonus. She also noted that, in making cultures from the tunnels around the beetle galleries, yeasts were always the first

organisms to appear, the blue-stain fungi only developing later. The different species of yeasts grew on sterilized pine on which the blue-stain fungus did not grow. The yeasts appear to have a stimulating effect on the staining fungus, allowing it to grow more vigorously on agar and to fruit more quickly than the pure cultures of Ceratastomella montium. Webb (1945) isolated Endomycopsis species that were constantly present in the tunnels, larvae and adults of the wood-boring Australian ambrosia beetles.

PURPOSE OF INVESTIGATION

The ultimate purpose of this research project is to obtain information on the following points:

(1) Is the composition of the yeast flora, associated with each bark-beetle species, relatively constant with respect to:

(a) geographical distribution

(b) different stages of the life cycle

(c) different host species which the beetles might attack

(2) Does each yeast flora produce an attrahent specific only for its associated bark beetle species, or rather is it attractive to many species?

(3) Can the attrahents be ultimately produced and used in a direct control method?

(4) Is the composition of the yeast flora at all critical in determining the success or failure of brood development in different host species which a given insect species might attack?

The answers to these questions are indispensible to an understanding of host selection, host resistance, attraction and bark beetle biology.

The initial objective of the ~~project~~ investigation was to isolate and identify the yeasts associated with each of the metamorphic stages of some of the more important bark beetles removed from different trees.

RESULTS AND DISCUSSION

A total of one hundred and fifty six ~~beetles~~ individuals were dissected. One hundred and

forty-eight yeasts, seven bacteria, two molds and one actinomycete were isolated and are now being identified. Twenty-seven of the dissected beetles did not yielded any micro-organisms. Of these, twenty were adults, two pupae, three larvae and one adult beetle in flight. Table I summarizes the number of beetles dissected at the different stages in the life cycle with the corresponding number of yeasts isolated.

From the one hundred and forty-eight yeasts isolated, and partially identified, three main groups emerged. The first group, designated tentatively as Hansenula A, contains thirty-five yeasts. All of these ferment glucose, assimilate glucose and maltose, and utilize nitrate as the sole nitrogen source. The species of beetle and the corresponding trees from which this group of yeasts was isolated are listed in Table II. The second and third groups designated tentatively as Yeast B and Yeast C contain fifty-three and twelve isolates respectively. These are listed in Tables III and IV respectively, in the same manner as the first group. From the above data it appears that Hansenula A is more prevalent in Dendroctonus species. Yeast B seems to be more common in Ips species, whereas Yeast C seems to be equally distributed between the two bark-beetle genera. A summary of these results is tabulated in Table V. The remaining forty-seven yeasts, which do not fall in the three large groups, are, apparently, single species (will be called throughout this paper as Yeast SP) based on information thus far obtained. From the latter group twenty-three are nitrate positive and twenty-four negative. Both yeasts, Hansenula A and Yeast B can utilize nitrate as the sole nitrogen source. These two groups together with the twenty-three yeasts from Yeast SP group make up one hundred eleven yeasts out of one hundred forty-eight total isolates that can utilize nitrate. This is of considerable interest because, in most yeast surveys reported in the literature, the number of nitrate positive yeasts is very small.

Only in the case of D. monticolae in western white pine and ponderosa pine, and Ips oregonei in jeffrey pine were beetles examined for their yeast flora, from four

life cycle stages, namely, larvae, pupae, callow-adults and adults. The yeast flora of all these beetles was compared in the different stages of the life cycle. In addition, in the case of D. monticolae from western white pine three beetles were caught in flight and their intestinal microflora isolated. Tables VI, VII and VIII list the different yeast groups found in the larvae, pupae, callow-adults and adults. From Tables VI and VII we can see that the yeasts belonging to the four different tentative groups are fairly well distributed in the different stages of the life cycle of D. monticolae isolated from western white and ponderosa pines. In Table VIII we see that all the stages of Ips oregonei contain Yeast B but more significant is the fact that Hansenula A and Yeast C were not found in this species of bark beetle. To confirm this interesting finding it would be desirable to dissect more beetles in the different stages to determine whether their yeast flora is the same.

SUMMARY

(1) The yeast flora is relatively specific in the different stages of the life cycle of Ips oregonei from Jeffrey pine, but it is rather variable in D. monticolae from ponderosa and western white pines.

(2) The yeast flora of each bark beetle species is relatively constant with respect to the different host species which the beetles attack. The findings with regard to the yeast flora found could be reproduced in separate experiments.

TABLE I

Number of beetles dissected in the different stages of their life cycle, and the corresponding number of yeasts isolated.

Stage in Life Cycle	No. of individuals Dissected	No. of individuals Yielding no growth	No. of Yeasts isolated
Adults in Flight	4	1	3
Adults Before Emergence	98	21	94
Callow-Adults	15	0	16
Pupae	17	2	15
Larvae	22	3	19
Total	156	27	148

TABLE II

Number of yeasts belonging to Hansenula A, isolated from Dendroctonus and Ips beetles

Species of Tree	Species of Beetle	No. of Isolated Hansenula A
P. jeffreyi	D. jeffreyi	6
P. ponderosa	D. monticolae	17
P. Monticola	D. monticolae	7
P. contorta var. latifolia	D. monticolae	3
P. ponderosa	Ips. confusus	1
P. ponderosa	Ips. oregoni	1
		TOTAL 35

TABLE III

Number of Yeasts belonging to Yeast B, isolated from Dendroctonus and Ips beetles.

Species of Tree	Species of Beetle	No. of Isolated Yeast B
P. monticola	Ips cŭnfusus	1
P. lambertiana	Ips cŭnfusus	1
P. ponderosa	Ips cunfusus	2
P. jeffrey	Ips imarginata	6
P. jeffrey	Ips oregonĕi	17
P. ponderosa	Ips oregonĕi	2
P. contorta var. latifolia	Ips oregonĕi	8
P. jeffrey	D. jeffrey	2
P. contorta var. latifolia	D. monticolae	3
P. ponderosa	D. monticolae	6
P. monticola	D. monticolae	5
	TOTAL	53

TABLE IV

Number of Yeasts belonging to Yeast C, isolated from Dendroctonus and Ips beetles.

Species of Tree	Species of beetle	No. of Isolated Yeast C.
P. ponderosa	D. monticolae	2
P. monticola	D. monticolae	5
P. contorta var. latifolia	Ips oregoni	1
P. monticola	Ips confusus	2
P. ponderosa	Ips confusus	2
	TOTAL	12

TABLE V

Number of yeasts isolated from Dendroctonus and Ips beetles, belonging to Hansenula A and Yeast C

Species of Tree	Species of Beetle	No. of Isolated Hansenula A	No. of Isolated Yeast B	No. of Isolated Yeast C	Total No. of Isolates
P. jeffrey	D. valens	0	1	0	1
P. jeffrey	D. jeffrey	6	2	0	17
P. contorta var. latifolia	D. monticolae	3	3	0	8
P. ponderosa	D. monticolae	17	6	2	39
P. monticola	D. monticolae	7	5	5	22
	TOTAL	33	17	7	87
P. monticola	Ips confusus	0	1	2	4
P. lambertiana	Ips confusus	0	1	0	1
P. ponderosa	Ips confusus	1	2	2	9
P. jeffrey	Ips imarginata	0	6	0	7
P. jeffrey	Ips oregoni	0	17	0	19
P. ponderosa	Ips oregoni	1	2	0	6
P. contorta var. latifolia	Ips oregoni	0	8	1	13
	TOTAL	2	37	5	59

TABLE VI

Number of yeasts isolated from *D. monticolae* from *P. monticola* belonging to each of the four tentative groups

Stage in Life Cycle	Hansenula A	Yeast B	Yeast C	Yeast SP	Total Yield of Yeasts
Larvae	0	2	1	1	4
Pupae	1	2	0	1	4
Callow-Adults	4	1	1	0	6
Adults	2	0	3	0	5
Beetles in Flight	0	0	2	1	3
Total	7	5	7	3	22

TABLE VII

Number of yeasts isolated from D. monticolae from P. pondorosa belonging to each of the four tentative groups

Stage in Life Cycle	Hansenula A	Yeast B	Yeast C	Yeast SP	Total Yield of Yeasts
Larvae	0	2	0	3	5
Pupae	3	1	0	1	5
Callow Adults	2	1	1	1	5
Adults	3	1	0	1	5
Total	8	5	1	6	20

TABLE VIII

Number of yeasts, belonging to each of the four tentative groups, isolated from Ips oregonei from P. jeffreyi.

Stage in Life Cycle	Hansenula A	Yeast B	Yeast C	Yeast SP	Total yield of yeasts
Larvae		5			5
Pupae		3		2	5
Callow-Adults		5			5
Adults		4			4
Total		17		2	19

REFERENCES

Baumberger, J. P. 1919 — A nutritional study of insects, with special reference to microorganisms and their substrata. J. Exp. Zool. 28: 1-83.

Beck, O. 1922 — Cited by Mrak, E. M., and Phaff, H. J. (1948) in Yeasts. Rev. Ann. Microbiology.

Caird, R. W. 1934 — Physiology of pines infested with bark beetles. Bot. Gaz. 96: 707-733.

Callaham, R. Z. 1952 — Personal communications

El-Tabey, A.S.A.M. 1950 — Ph D. thesis University of California

Fawcett, H. S. 1929 — Nematospora on pomegranetes, citrus and cotton in California. Phytopathology 19: 479-482.

Gordon, Aaron 1933 — Apparatus used in a study of the western pine beetle. U.S.D.A. Forest Service. Bur. of Ent. Typed Report.

Grossman, H. 1930 — Contribution to the knowledge of the life partnership between bark beetles and fungi. (Translated by Rumbold, C.) Zeitch. fur Parasitenkunde 3: 56-102.

Holst, E. C. 1936 — Zygosaccharomyces pini, a new species of yeast associated with bark beetles in pines. J. Agr. Res. 53: 513-518.

Holst, E. C. 1937 — Aseptic rearing of bark beetles. J. Econ. Ent. 30:677.

Hopkins, A. O. 1909 — Bark beetles of the genus Dendroctonus. U.S.D.A. Bureau of Ent. Bull. 83. Part I.

Jeffrey, R. N. 1931 — The concentration of certain sugars in the bark of the western yellow pine in relation to western pine beetle attraction. Final Report. U.S.D.A. Bureau of Ent. and Plant Quarant. Insect Labs. University of Calif., Berkeley.

Keen, F. P. 1951 — Forest insect problems of California. Report of U.S.D.A., Bur. of Ent. and Plant Quarnt. Forest insect Labs. University of Calif., Berkeley.

Leach, J.G. 1940 — Insect transmission of plant diseases. McGraw Hill Pub. Co., N. Y.

Leach, T., Orr, L.W. & Christensen, C. 1934 — The interrelationships of bark beetles and blue-staining fungi in felled Norway pine. J. Agr. Res. 49:315-341.

Mrak, E. M. & Phaff, H. J. 1948 — Yeasts. Ann. Rev. of Microbiology. 1-46.

Person, H. L. 1926 — Western pine beetle investigations. Report No. 5 Host selection. Report No. 5 Attraction studies with the western pine beetle. U.S.D.A., Bur. of Ent. & Plant Quarnt. Insect Labs., University of Calif., Berkeley.

Person, H. L. 1927 — Prelimenary study of the microorganisms associated with the western pine beetle. U.S.D.A., Bur. Ent. and Plant Quarant. Insect Labs. University of Calif., Berkeley. Typed report.

Person, H. L. 1931 — Theory in explanation of the selection of certain trees by the western pine beetle. J. Forestry 29: 696-699.

Rumbold, C. T. 1941 — A blue-stain fungus, Ceratostomella montium n. sp. and some yeasts associated with two species of Dendroctonus. J. Agr. Res. 62:589-601.

Schneider-Orelli 1913 — Cited by Steinhaus (1946) in Insect Microbiology.

Siemaszko, W. 1939 — Fungi associated with bark beetles in Poland. Plantapolon 7:54 pp.

Steinhaus, E. A. 1946 — Insect microbiology. Comstock Pub. Co., Inc. Ithaca, N.Y.

Stuble, G. R. 1930 — Prelimenary studies of the feeding habits of adult western pine beetles. U.S.D.A., Bur. Ent. and Plant Quarant. , Forest Insect Lab., University of Calif., Berkeley. Typed reports.

Uvarov, B.P. 1929 — Insect nutrition and metabolism. The Trans. Ent. Soc., London LXXVI Part II.

Verrall, A.F. 1943 — Fungi associated with certain ambrosia beetles. J. Agr. Res. 66: 135-144.

Wallace, G. B. 1932 — Coffee bean disease. Relation of Nematospora gossypii to the disease . Trop. Agr. (Trinidad) 9: 127.

Wingard, S. A. 1925 — Studies on pathogenicity, morphology, and cytology, of N. phaseoli. Cited by Steinhaus 1946, in Insect Microbiology.

Webb, S. 1945 — Australian Ambrosia fungi. Proc. Roy. Soc. Vic. 57:57-80.